

# CHROMOSOME STUDIES ON FISH OF THE SUBORDER NOTOTHENIOIDEI COLLECTED IN THE WEDDELL SEA DURING EPOS 3 CRUISE

by

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**ABSTRACT.** - Twenty-six species belonging to the suborder Notothenioidei (8 Nototheniidae, 8 Channichthyidae, 4 Bathydraconidae and 6 Artedidraconidae) have been karyotyped. The results are listed together with all other karyotypic data now available for the suborder. Intraspecific variation of karyotypic numbers and formulae is not significant and confirms that within a species, the karyotype is highly stable. In Nototheniidae ( $2n = 22-50$ ) and Bathydraconidae ( $2n = 36-48$ ), chromosome numbers show a strong interspecific variability involving inversions, Robertsonian translocations and probably duplications. In Channichthyidae ( $2n = 48$ ), the chromosome number is conserved and the differences in chromosome structure are correlated with peri- or paracentric inversions and also duplications. In Artedidraconidae, the karyotype ( $2n = 46$ ) appears more generic than specific with minor restructuring. Morphologically differentiated sex chromosomes have been recorded in 5 species of Channichthyidae ( $X_1X_1X_2X_2X_1X_2Y$ ) and also appears in one Bathydraconidae ( $XX.XY_1Y_2$ ).

**RÉSUMÉ.** - Les caryotypes de 26 espèces appartenant au sous-ordre des Notothenioidei (8 Nototheniidae, 8 Channichthyidae, 4 Bathydraconidae and 6 Artedidraconidae) ont été étudiés et réunis avec tous ceux qui avaient déjà été obtenus pour le sous-ordre. La comparaison des nombres diploïdes et des formules chromosomiques avec ceux de certaines espèces déjà étudiées dans d'autres secteurs ne révèle pas de variation intraspécifique significative et confirme ainsi la stabilité du critère caryotypique dans ces familles. Chez les Nototheniidae ( $2n = 22-50$ ) et les Bathydraconidae ( $2n = 36-48$ ), la variabilité interspécifique du caryotype est importante et met en jeu différents types de restructurations (inversions, translocations robertsoniennes) et très vraisemblablement des duplications. Chez les Channichthyidae ( $2n = 48$ ) le nombre diploïde de chromosomes est conservé et la variabilité interspécifique ne met en jeu que des inversions para- et péri-centriques et des duplications. Chez les Artedidraconidae, le caryotype ( $2n = 46$ ) apparaît plus générique que spécifique avec peu de remaniements. L'existence de chromosomes sexuels multiples morphologiquement différenciés est signalée chez 5 Channichthyidae ( $X_1X_1X_2X_2X_1X_2Y$ ) et est très probable chez un Bathydraconidae ( $XX.XY_1Y_2$ ).

Key-words. - Notothenioidei, PSW, Weddell Sea, Antarctica, Cytogenetics, Chromosomes.

In both numbers and biomass, the Antarctic ichthyofauna is dominated by species of the suborder Notothenioidei. The taxonomy and phylogenetic relationships within and among the families of this suborder remain controversial. For example, surveys of various morphological and osteological characters have lead to the division of the suborder into five (Iwami, 1985) and more recently, six families (Hureau, 1985, 1986). The largest and most diversified family, Nototheniidae, is considered to be monophyletic, but within the family, generic classification is still uncertain (Andersen and Hureau, 1979; Andersen, 1984; Balushkin, 1984; Voskoboinikova, 1986; DeWitt *et al.*, 1990). In particular, the

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specific composition of the nototheniid genus *Trematomus* is not clear (Andersen, 1984; Balushkin, 1982, 1984; DeWitt *et al.*, 1990).

Karyological studies have been undertaken in different sectors of the Southern Ocean (Prirodina, 1984, 1986, 1989; Prirodina and Neyelov, 1984; Doussau de Bazignan and Ozouf-Costaz, 1985; Phan *et al.*, 1986, 1987; Ozouf-Costaz 1987a and b) in order to bring a new criterion for elucidating generic phylogeny. Results from these studies concerned scattered species (1 Bovichthyidae, 1 Harpagiferidae, 1 Bathydraconidae, 7 Channichthyidae and 17 Nototheniidae) mostly collected from subantarctic waters, no data was available for species of the family Artedidraconidae. Preliminary analysis showed that while Channichthyidae had a conservative karyotype with little variation in chromosome morphology, Nototheniidae exhibited a wide interspecific chromosome variability involving many types of morphological and numerical changes.

To estimate this variability within the families, a programme to examine nototheniid fish cytogenetics was developed for the EPOS cruise on board the R.V. "Polarstern" in the inner Weddell Sea (January-March 1989). The Weddell Sea, allocated to the East Antarctic Province (DeWitt, 1971) includes at least 38 Notothenioidei (Ekau, 1990), the taxonomy of the species belonging to the genus *Pogonophryne* remaining under study; by 1988, karyotypes of only six of these 38 species were recorded from other sectors of the Southern Ocean. The objectives of the program were:

- to obtain the standard karyotypes of as many species as possible belonging to this suborder;
- to compare the fish species composition of the Weddell Sea to those from other High Antarctic areas previously or simultaneously investigated (Prydz Bay, Ross Sea...) particularly from the cytotaxonomical viewpoints and to estimate possible intraspecific variations in the karyotypes;
- to test the value of the karyotypic criterion as a specific or generic character within each family;
- to estimate whether numerical or morphological changes and alterations in karyotype can be interpreted in terms of evolution. If interpretation was possible, could karyotypes provide enough information for being used alone or combined with other characters (i.e., morphological, biochemical, molecular...) as a means of a phylogenetic analysis;
- to detect any abnormality, polymorphism, peculiarities such as supernumerary chromosomes, morphologically distinguishable sex chromosomes, polyploidy, which may be suitable for a more detailed study.

## MATERIALS AND METHODS

Live fish were collected by three fishing methods, Agassiz trawls, semi-pelagic trawls and ground trawls. Samples caught with Agassiz trawls and semi-pelagic trawls showed less species diversity but the condition of the fish was better than from ground trawls. Chromosome preparations and Giemsa staining followed the procedures described by Doussau de Bazignan and Ozouf-Costaz (1985) with the following modifications: specimens were kept alive in well oxygenated sea water tanks at +3°C; Colchicine (0.5%) was injected intraperitoneally at 0.3 ml per 100 g weight; fish were sacrificed between 4 and 24 h after injection, according to their survival ability in aquariums. Kidney was homogenised directly in the hypotonic KCl solution and the suspension hypotonised at 0°C for 50 mn. Chromosome counts were obtained either from photographs or directly by microscope (100X). Designation of chromosome types followed the criteria of Levan *et al.* (1964). m: metacentrics, sm: submetacentrics, a: acrocentrics, s: satellites or achromatic region, B: microchromosomes or supernumerary chromosomes. When mounting the karyograms, the chromosomes were not grouped according to their



types; we preferred to present them in order of decreasing size and paired by eye. Although this method could be considered to be subjective, it can provide information about the relative place of some marker chromosomes in the karyogram. In addition, it allows easy identification of unpaired chromosomes, when present, or complete heteromorphic pairs. This also facilitates the comparison between species karyotypes. On the figures, scale bars represent 10  $\mu\text{m}$ . Except when indicated in the text, at least 20 metaphase plates per specimen were examined. For species having identical karyotypes in both sexes, the karyogram of the best metaphase spread is produced. For previously published karyotypes, a figure is provided only if we have obtained better or different results. Silver staining of the nucleole organizer regions (NORs), was attempted on species providing sufficient metaphase plates, according to the method of Howell and Black, 1980.

For each species reference specimens of the karyotype were preserved in the Museum national d'Histoire naturelle of Paris fish collection. For *Nototheniidae* the nomenclature used in the text, table and figures is that adopted by DeWitt *et al.* (1990).

#### **Nototheniidae**

- Dissostichus mawsoni* Norman, 1937: one female, MNHN 1990-1307.  
*Trematomus scotti* (Boulenger, 1907): one female, MNHN 1990-1281.  
*Trematomus eulepidotus* Regan, 1914: three males, MNHN 1990-1370 to -1372.  
*Trematomus lepidorhinus* (Pappenheim, 1911): one male, MNHN 1990-1355; one female, MNHN 1990-1360.  
*Trematomus pennellii* Regan, 1914: one male, MNHN 1991-564; one female, MNHN 1991-565.  
*Trematomus hansonii* Boulenger, 1902: one female, MNHN 1990-1327.  
*Trematomus bernacchii* Boulenger, 1902: one male, MNHN 1991-561.  
*Pleuragramma antarcticum* Boulenger, 1902: three males, MNHN 1991-572 to -574; 2 females, MNHN 1990-575 and -576.

#### **Channichthyidae**

- Chionodraco myersi* DeWitt and Tyler, 1960: two males, MNHN 1990-1104 and -1109; three females, MNHN 1990-1103, -1108 and -1113.  
*Chionodraco hamatus* (Lönnberg, 1905): two females, MNHN 1990-1093 and -1100.  
*Chaenodraco wilsoni* Regan, 1914: one female and one male, both MNHN 1990-1122.  
*Chionobathyscus dewitti* Andriashev and Neyelov, 1978: one male, MNHN 1990-1073.  
*Neopagetopsis ionah* Nybelin, 1947: two males, MNHN 1990-1063, -1064; one female, MNHN 1990-1078.  
*Pagetopsis macropterus* (Boulenger, 1907): one male, MNHN 1990-1139.  
*Pagetopsis maculatus* Barsukov and Permitin, 1958: one female, MNHN 1990-1142.  
*Cryodraco antarcticus* Dollo, 1900: five males, MNHN 1990-1087, -1079 to -1081 and -1083; three females, MNHN 1990-1082 and -1092.

#### **Bathydraconidae**

- Bathydraco marri* Norman, 1938: one male, MNHN 1990-1215; one female, MNHN 1990-1214.  
*Racovitzia glacialis* Dollo, 1900: one female, MNHN 1990-1218.  
*Gerlachea australis* Dollo, 1900: two females, MNHN 1990-1199 and -1203.  
*Cynodraco mawsoni* Waite, 1916: one female, MNHN 1990-1191.

### Artedidraconidae

*Artedidraco orianae* Regan, 1914: one female, MNHN 1990-1238; two males, MNHN 1990-1239, -1240.

*Artedidraco shackletoni* Waite, 1911: one female, MNHN 1990-1248; one male, MNHN 1990-1246.

*Pogonophryne barsukovi* Andriashev, 1967: four females, MNHN 1991-544 to -548; four males, MNHN 1991-543, -545, -549, -551.

*Pogonophryne marmorata* Norman, 1938: three males, MNHN 1991-540, -542 and -550.

*Pogonophryne scotti* Regan, 1914: four females, MNHN 1991-552, -553, -555 and -557.

*Pogonophryne mentella* Andriashev, 1967: one male, MNHN 1991-541; one female, MNHN 1991-558.

## RESULTS

Although most species provided positive results, more technical difficulties were encountered than with subantarctic species. Considerable differences were observed in the proportion of metaphase spreads in our preparations, the mitotic index was often very low. This is possibly linked to the low metabolism of these fish, their fragility and inability to survive more than a few hours after colchicine injection. For these reasons, our attempts with a single specimen of *Dacodraco hunteri*, two specimens of *Aethotaxis mitopteryx*, and one of *Akarotaxis nudiceps* ended in failure. We also had difficulties removing the kidneys of smaller species (of Nototheniids and Artedidraconids) and the use of alternative tissues (spleen, gills...) in replacement did not produce any evidence of dividing cells. The best mitotic rates were obtained with species treated with colchicine for more than 15 hours. The absence of one sex in many species karyotyped, and the small number of specimens studied for some species, make some results more informative than statistical.

### Nototheniidae

*Dissostichus mawsoni*. Seventeen complete metaphase plates were examined from the single female, revealing a diploid number  $2n = 48$ , (fundamental number  $FN = 52$ ) consisting of  $2m + 2sm + 44a$  chromosomes of slowly decreasing size (Fig. 1). This makes difficult the accurate identification of the pairs, the  $m$  being one of the smallest. However the karyotype appears to be very similar to that of the other species of the genus, *Dissostichus eleginoides* (Doussau de Bazignan and Ozouf-Costaz, 1985).

*Trematomus scotti*. A single female produced only two metaphase spreads of poor quality, so this result is given only for information. The diploid number,  $2n = 50$ , consists of  $4m + 2-4sm + 42-44a$  (Fig. 2). One chromosome of the smallest  $m$  pair carries an achromatic region which may well correspond to the nucleole organizer regions (NORs).

*Trematomus eulepidotus*. The karyotype of this species has already been studied in Prydz Bay (Ozouf-Costaz and Doussau de Bazignan, 1987) but our results for the three males from the Weddell Sea are better and slightly different:  $2n = 24$  ( $20m + 4sm$ ),  $FN = 48$ . The smallest  $m$  pair also carries an achromatic region (Fig. 3). The diploid number obtained earlier was the same. The formula ( $8m + 14sm + 2a$ ),  $FN = 46$  and the distribution of the  $m/sm$  pairs can be discussed, but not the presence of a large acrocentric pair, possibly derived from a pericentric inversion in a previously large metacentric. More specimens need to be karyotyped in both geographical sectors in order to establish whether these differences are due to a widespread chromosomal polymorphism or characterize



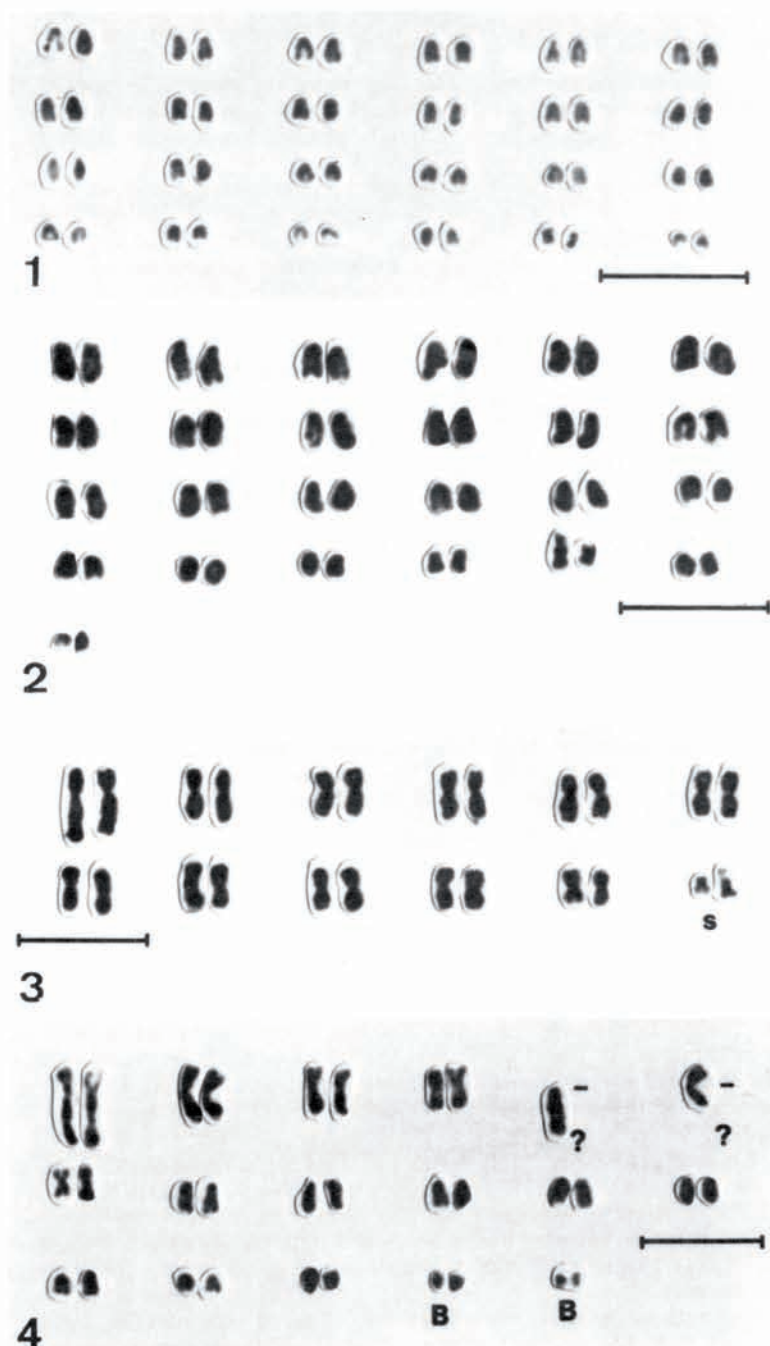


Fig. 1. - Karyotype of *Dissostichus mawsoni* female. Fig. 2. - Karyotype of *Trematomus scotti*. Fig. 3. - Karyotype of *Trematomus eulepidotus* male. Fig. 4. - Karyotype of *Trematomus pennellii* male.

two distinct populations. The smallest m pair also carries an achromatic region (Fig. 3).

*Trematomus lepidorhinus*. The karyotypes are identical in male and female and the chromosome formula,  $2n = 48$ , ( $4m + 44a$ ) corresponds with the results obtained for this species in Prydz Bay (Ozouf-Costaz and Doussau de Bazignan, 1987).

*Trematomus pennellii*. Five metaphase plates were examined for the male and 9 for the female. The female spreads were of lesser quality. Chromosome numbers were identical in both sexes,  $2n = 32$ , but we were unable to prepare a karyogram for the female. The male chromosome formula (Fig. 4),  $9m + 2sm + 17a + 4B$ , showed considerable size differences. The two smallest pairs of chromosomes were identified as "B", because it evokes the so-called supernumerary microchromosomes. We noted the presence of two heteromorphic chromosomes (?) of rather equal size, one being metacentric and the other acrocentric.

*Trematomus bernacchii*. For the single male studied, our results differ slightly from those obtained by Phan *et al.* (1986) for specimens from the South Shetland Islands. The chromosome numbers,  $2n = 48$ , are identical but our formula ( $2m + 2sm + 44a$ ) includes an additional pair of sm chromosomes (see Fig. 5). This difference may be due to the quality of preparations. The chromosome size is slowly decreasing and one chromosome of the m pair, possibly the smallest, also carries an achromatic region.

*Trematomus hansonii*. We were able to collect only one female and obtained the same karyotype  $2n = 48$  ( $2m + 4sm + 42a$ ) as Phan *et al.* (1986) for South Shetland specimens.

*Pleuragramma antarcticum*. Even though this species is pelagic and might be expected to be fragile, it was found to be robust when subjected to standard treatments and five specimens were able to survive in aquariums up to 10 hours after colchicine injection. This treatment was sufficient to produce a very high mitotic index. About 75 metaphase plates were examined, providing identical results in males and females:  $2n = 48$  ( $8m + 22sm + 8a$ ),  $FN = 68$  chromosomes of slowly decreasing size (Fig. 6).

### Channichthyidae

In the Weddell Sea, the karyotypes of males and or females of eight species of Channichthyidae were studied. Male heterogamety linked to the possible existence of a multiple sex chromosome system in about five species of this family was revealed. In another study by Italian colleagues complementary results were obtained in the Ross Sea. Conclusions concerning this evident heterogamety together with hypotheses on the different types of mechanisms involved (Morescalchi *et al.* in press) are in a collaborative publication. Here we only deal with the standard karyotypes of Channichthyids obtained during EPOS cruise and the existence of heteromorphic sex chromosomes is briefly mentioned but not analysed.

*Chionodraco myersi*. Only females of this species had been karyotyped from Prydz Bay (Ozouf-Costaz, 1987a). Females studied in the Weddell Sea also have a diploid chromosome number  $2n = 48$  ( $2m + 6sm + 40a$ ),  $FN = 56$  (Fig. 7a). Male karyogram (Fig. 7b) revealed the presence of a multiple sex chromosome system of the type  $X_1X_2Y$  (female  $X_1X_1X_2X_2$ ). Satellites are carried by the short arms of the smallest sm pair.

*Chionodraco hamatus*. We were able to study only two females:  $2n = 48$  ( $2m + 4sm + 42a$ ),  $FN = 54$  (Fig. 8a). The size of these chromosomes is slowly decreasing from  $2.5 \mu m$  to  $1 \mu m$ , the largest pair being submetacentric. As in *C. myersi*, satellites are carried by the short arms of the smallest sm pair and correspond to the NORs (Fig. 8b). Moreover, after Ag-staining, the distal part of a large acrocentric is also positively stained, but we did not find any homolog (see double

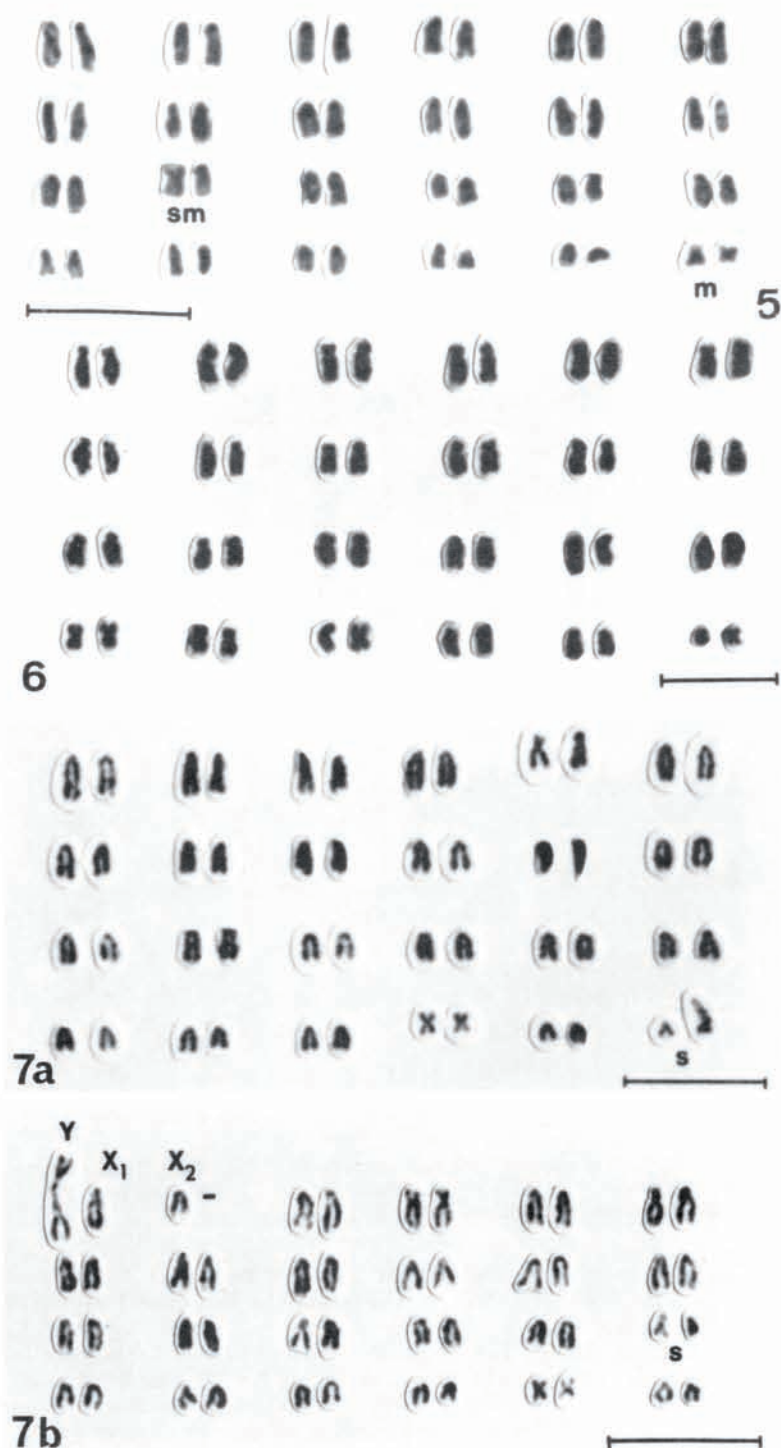


Fig. 5. - Karyotype of *Trematomus bernacchii* male. Fig. 6. - Karyotype of *Pleuragramma antarcticum*. Fig. 7a. - Karyotype of *Chionodraco myersi* female. Fig. 7b. - Karyotype of *Chionodraco myersi* male.



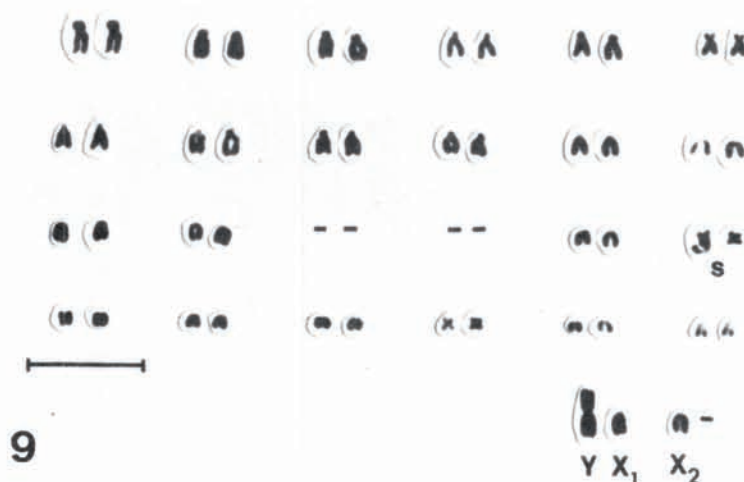
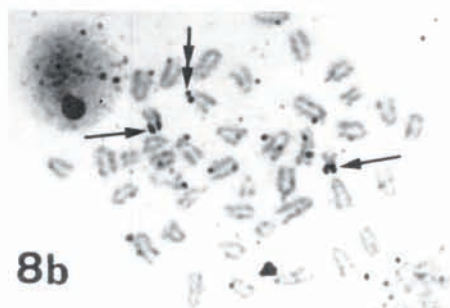
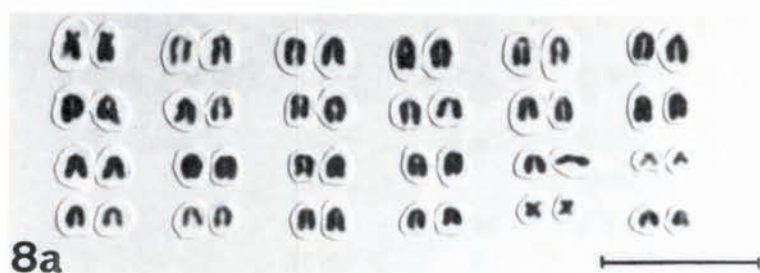
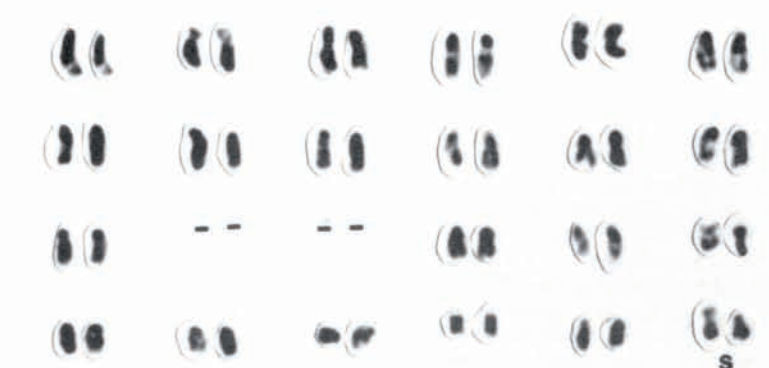


Fig. 8a. - Karyotype of *Chionodraco hamatus* female. Fig. 8b. - Silver-staining of the nucleole-organizer regions (NORs) in the chromosomes of *Chionodraco hamatus*. Fig. 9. - Karyotype of *Chaenodraco wilsoni* male.

arrow, Fig. 8b). In males, a multiple sex chromosome system of the type  $X_1X_2Y$  has been simultaneously recorded by Morescalchi *et al.* (1991) with specimens from the Weddell Sea.

*Chaenodraco wilsoni*. Females of this species had been karyotyped in Prydz Bay (Ozouf-Costaz, 1987a). The female studied in the Weddell Sea has  $2n = 48$  ( $4m + 6sm + 38a$ ),  $FN = 58$ . The male also exhibits a multiple sex chromosome system of the type  $X_1X_2Y$  (with  $Y$  more metacentric than in *C. myersi*, and  $X_1$  and  $X_2$  acrocentric). Satellites are carried by one of the smallest pairs of metacentrics (Fig. 9).

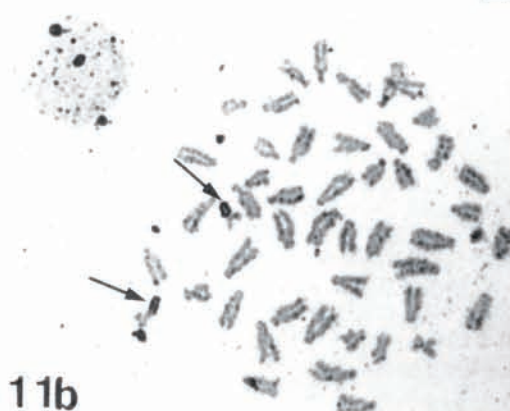




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11a



11b

Fig. 10. - Karyotype of *Chionobathyscus dewitti* male. Fig. 11a. - Karyotype of *Neopagetopsis ionah*. Fig. 11b. - Silver-staining of the nucleole-organizer regions (NORs) in the chromosomes of *Neopagetopsis ionah*.

*Chionobathyscus dewitti*. Only ten metaphase plates were examined for the single male studied, but the karyotype is  $2n = 47$  ( $m + 4.6sm + 38-36a$ ) (Fig. 10) with three heteromorphic chromosomes, the morphology of which is very similar to the system  $X_1X_2Y$  of *C. wilsoni* ( $Y$  very large and metacentric). Satellites can be seen on a little  $sm$  pair and have unequal sizes.

*Neopagetopsis ionah*. The two males and single female karyotyped provided abundant and identical results:  $2n = 48$  ( $2m + 8sm + 38a$ ),  $FN = 58$ . As in other species, chromosome size is slowly decreasing (between 3 and 1  $\mu m$ ), one of the largest pairs being a submetacentric. Nearly all the short arms of acrocentric chromosomes are visible, which is not the case in the karyograms of the other Channichthyids described here (Fig. 11a). The satellites carried by the smallest  $sm$  pair are positively Ag-stained and well correspond to the NORs-bearing chromosomes (Fig. 11b).

*Pagetopsis macropterus*. We were able to study a single male in poor condition. Only two metaphase spreads allowed chromosome counts,  $2n = 47$ : Their examination reveals a higher proportion of  $m-sm$  elements than in other Channichthyids, and a very large  $sm$  unpaired element that obviously corresponds to the  $Y$  chromosome described by Morescalchi *et al.* (in press.) from two specimens caught in the Ross Sea.

*Pagetopsis maculatus*. We were unable to prepare a karyogram for the single female karyotyped and provide its chromosome number,  $2n = 48$ , and formula (approximately  $2m + 6sm + 40a$ ) for information only, based on the examination of five metaphase plates.

*Cryodraco antarcticus*. 85 metaphase plates were examined, revealing identical karyotypes for males and females:  $2n = 48$  ( $2m + 4sm + 42a$ ),  $FN = 54$ . Satellites are beared by the short arms of the smallest  $sm$  pair (Fig. 12) and have unequal lengths. The chromosome sizes decrease progressively, ranging between 2.5 and 1  $\mu m$ , the largest pair being  $sm$ .

### Bathydraconidae

*Bathydracono marri*. All metaphase plates studied in the female showed a diploid number of  $2n = 38$  ( $4sm + 34a$ ) (Fig. 13a). The largest  $sm$  pair measures 5-6  $\mu m$ , the smallest about 1  $\mu m$ . The serie of acrocentrics gradually decreases in size. The male was found to be heterogametic:  $2n = 39$  ( $3sm + 36a$ ) (Fig. 13b), revealing the presence of a single large submetacentric (called  $X$ ) and two heteromorphic acrocentrics: a large one (called  $Y_1$ ) and a smaller one (called  $Y_2$ ), the size of which suggests that they are originating from the short and long arms of a previous  $X$  by fission.

*Racovitzia glacialis*. One female of this species had been already karyotyped from Prydz Bay (Ozouf-Costaz, 1990). The two females studied in the Weddell Sea exhibit the same chromosome number and formula:  $2n = 36$  ( $4m + 32a$ ),  $FN = 40$ .

*Gerlachea australis*. Two females were studied, providing a very low number of metaphase plates. Chromosome counts,  $2n = 48$  were obtained, but a single photograph made it possible to prepare a karyogram (Fig. 14) and establish the formula ( $2m + 2-4sm + 42-44a$ ). Satellites are carried by a small  $sm$  pair.

*Cygnodraco mawsoni*. A single female in poor condition provided 5 metaphase plates. Chromosome counts give a diploid number  $2n = 44-46$  acrocentrics. No  $m$  or  $sm$  shape could be detected.

### Artedidraconidae

#### Genus *Artedidraco*.

Two species were studied: *A. orianae* and *A. shackletoni*. In both species, the males and females exhibit the same karyotype:  $2n = 46$  ( $2m + 6sm + 40a$ ) (Fig. 15). Satellites are beared by the small metacentric pair.



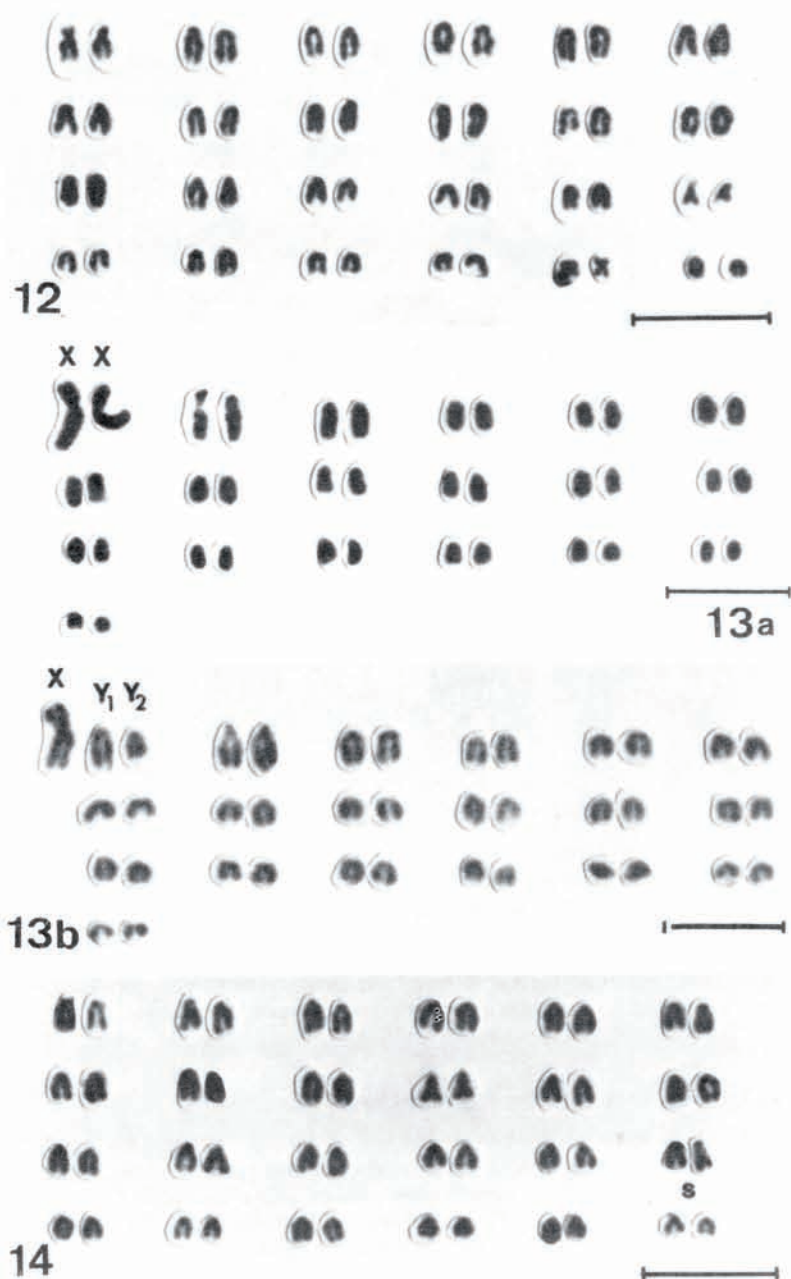


Fig. 12. - Karyotype of *Cryodraco antarcticus*. Fig. 13a. - Karyotype of *Bathhydraco marri* female. Fig. 13b. - Karyotype of *Bathhydraco marri* male. Fig. 14. - Karyotype of *Gerlachea australis* female.

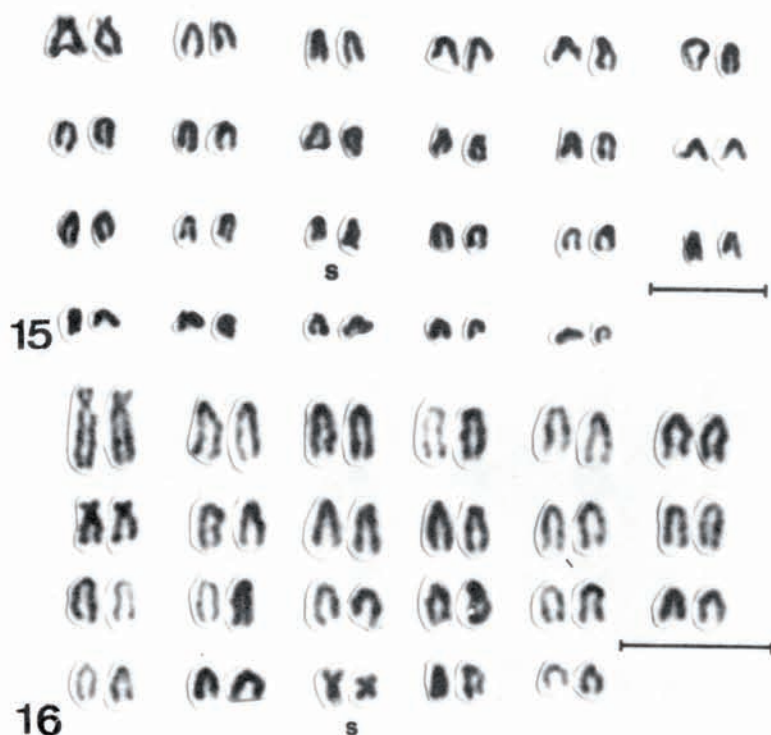


Fig. 15. - Karyotype of *Artedidraco orianae*. Fig. 16. - Karyotype of *Pogonophryne barsukovi*.

#### Genus *Pogonophryne*.

*P. barsukovi*, *P. marmorata*, *P. scotti*, and *P. mentella* were examined. All provided a high number of metaphase plates that were studied in detail. Neither the chromosome number,  $2n = 46$ , nor the morphological comparison of their chromosomes revealed any significant difference between these four species that exhibit identical formulas:  $2m + 4sm + 42a$ . As in *Artedidraco*, satellites are beared by the m pair and often have unequal sizes (see an example Fig. 16).

### DISCUSSION

Taking into account the karyotypic material from bibliography, data we had obtained earlier and during EPOS cruise and unpublished information collected by our colleagues (Table I), it is now possible to examine the degree of karyotypic diversity in four families of the suborder Notothenioidei: Channichthyidae, Nototheniidae, Bathydraconidae and Artedidraconidae, and to make hypotheses concerning the general characteristics of chromosomal evolution.

Figure 17 shows the histogram of relative frequency of diploid chromosome numbers in the suborder Notothenioidei. There is a strong prevalence of  $2n = 48$ . Ohno (1974) had proposed an ancestral karyotype of  $2n = 48$  uni-armed chromosomes for all perciform groups and this assumption seems to be quite valid for the Notothenioidei. Furthermore, the high frequency of diploid numbers lower than  $2n = 48$  suggest that many speciation events in these fish are associated with



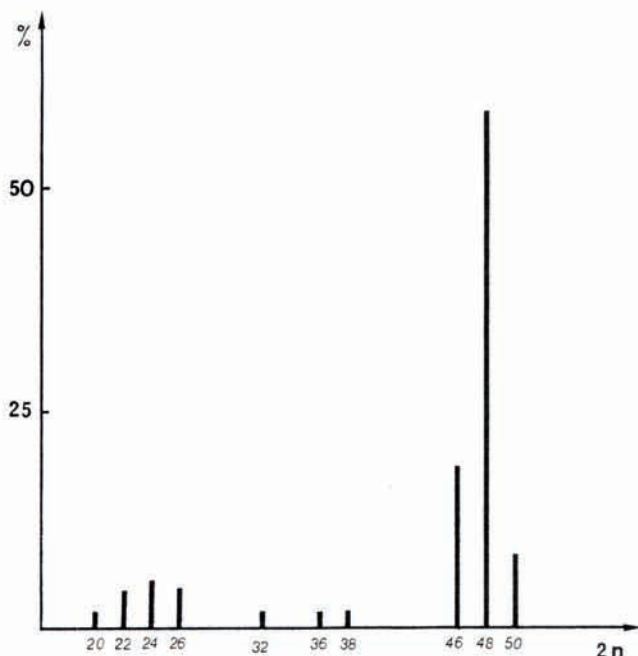


Fig. 17. - Relative frequency of diploid numbers ( $2n$ ) within the suborder Notothenioidei (51 species studied).

a decrease in chromosome numbers. However, when examining separately the karyotypic diversity in each family, it is obvious that every group has its own mode of chromosomal evolution.

#### Channichthyidae

Data are now available for all species of this family, except *Champscephalus esox* and the monotypic genus *Dacodraco hunteri*. For species that have been studied separately in different sectors of the Southern Ocean (*Chionodraco myersi*, *Chaenodraco wilsoni* in Prydz Bay and the Weddell Sea; *Pagetopsis maculatus* and *P. macropterus* in the Ross Sea and the Weddell Sea), no significant difference in chromosome numbers could be recorded, and only minor differences in chromosome morphology. This could indicate that there is no intraspecific variability in the karyotype, at least at the morphological level.

Chromosome diploid numbers,  $2n = 48$ , with a predominance of acrocentrics for all species, remain unchanged, suggesting a conservative type of karyoevolution. However, all species have different chromosome formulae, including 32 to 44 acrocentrics, FN ranging between 52 and 62. It is highly probable (as postulated by Prirodina, 1989) that the mechanisms involved in the FN increasing are pericentric inversions.

Another striking peculiarity is the frequent occurrence of multiple sex chromosome systems in at least five of these species, with the digametic males showing a  $X_1X_2Y$  and the homogametic females a  $X_1X_1X_2X_2$  system. As explained in Morescalchi *et al.* (in press), these multiple systems seem to have been acquired separately by each species and involve at least three different mechanisms. Their discovery adds a new apomorphic character to the morphological ones usually taken into account in phylogenetic studies.

Table 1. - Karyotype data on Notothenioidci.

Species	Locality	Sex	2n	Formula	Reference
<b>CHANNICHTHYIDAE</b>					
<i>Channichthys rhinoceratus</i>	Kerguelen	M/F	48	2m + 6sm + 40a	Doussau de Bazignan and Ozouf-Costaz, 1985
<i>Chamsocephalus gunnari</i>	Kerguelen	-	48	2m + 6sm + 44a	"
<i>Chamaocephalus aceratus</i>	South Sandwich Isl.	M	48	4m + 44a	Prirodina, 1989
<i>Chaenodraco wilsoni</i>	Prydz Bay	F	48	4m + 6sm + 38a	Ozouf-Costaz, 1987a
	Weddell Sea	M	47	5m + 6sm + 36a	Present paper
<i>Chionodraco rastrispinosus</i>	South Orkney Isl.	M	48	4m + 44a	Prirodina, 1989
<i>Chionodraco myersi</i>	Prydz Bay	F	48	2m + 6sm + 40a	Ozouf-Costaz, 1987a
	Weddell Sea	M	47	2m + 6sm + 39a	Present paper
<i>Chionodraco hamatus</i>	Ross Sea	M	47	2m + 4sm + 41a	Morescalchi et al. in press and
	Weddell Sea	F	48	2m + 4sm + 42a	Present paper
<i>Chionobathyscus dewitti</i>	Weddell Sea	M	47	5m + 4 - 6 sm + 38/36a	Present paper
<i>Pseudochaenichthys georgianus</i>	South Georgia	-	48	4m + 8sm + 36a	Prirodina, 1989
<i>Cryodraco antarcticus</i>	Weddell Sea	M	48	2m + 4sm + 42a	Present paper
	Ross Sea	F	48	2m + 4sm + 42a	Morescalchi et al. in press
<i>Neopagetopsis ionah</i>	Weddell Sea	M/F	48	2m + 8sm + 38a	Present paper
<i>Pagetopsis macropterus</i>	Ross Sea	F	48	2m + 12sm + 34a	Morescalchi et al. in press (Female and Male)
<i>Pagetopsis maculatus</i>	Weddell Sea	M	47	3m + 12sm + 32a	and present paper (Female)
	Weddell Sea	F	48	- 2m + 6sm + 40a	Present paper
<b>BOVICHTHYIDAE</b>					
<i>Cottoperca gobio</i>	Coasts of Chile, Falkland Islands	-	48 - 50	(48 - 50 a)	Prirodina, 1986
<b>HARPAGIFERIDAE</b>					
<i>Harpagifer antarcticus</i>	Signy Island	-	48	2m + 4sm + 42a	Ozouf - Costaz, unpub.
<b>BATHYDRACONIDAE</b>					
<i>Racovitzia glacialis</i>	Prydz Bay	F	36	4m + 32a	Ozouf - Costaz, 1990
<i>Gerlachea australis</i>	Weddell Sea	F	36	4m + 32a	Present paper
<i>Cygnodraco mawsoni</i>	Weddell Sea	F	48	2m + 2 - 4sm + 42 - 44a	Present paper
<i>Prionodraco evansii</i>	Weddell Sea	F	44 - 46	-	"
<i>Psilodraco breviceps</i>	?	-	20	-	Prirodina, comm. pers.
<i>Parachaenichthys georgianus</i>	?	-	48	48a	"
<i>Bathydraco marri</i>	Weddell Sea	M	39	3sm + 36a	Present paper
	"	F	38	4sm + 34a	"



ARTEDIIDRACONIDAE				
<i>Pogonophryne barsukovi</i>	Weddell Sea	M/F	46	2m + 4sm + 40a
<i>Pogonophryne marmorata</i>	"	M	46	"
<i>Pogonophryne scotti</i>	"	F	46	"
<i>Pogonophryne mentella</i>	"	M/F	46	"
<i>Artedidraco orianae</i>	"	M/F	46	2m + 6sm + 38a
<i>Artedidraco shackletoni</i>	"	M/F	46	"
NOTOTHENIIDAE				
<i>Dissostichus eleginoides</i>	Kerguelen	-	48	2m + 2sm + 44a
<i>Dissostichus mawsoni</i>	Weddell Sea	F	48	2m + 2sm + 44a
<i>Patagonotothen longipes</i>	Magellanic Region	M/F	48	2m + 46a
<i>Patagonotothen ramsayi</i>	"	-	48	2m + 2sm + 44a
<i>Gobionotothen gibberifrons</i>	South Shetland Isl.	-	46	4m + 2sm + 40a
<i>Gobionotothen acuta</i>	Heard Island	F	50	6m + 8sm + 32a + 4B
<i>Lepidonotothen kempi</i>	Prydz Bay	M/F	48	4sm + 44a
<i>Lepidonotothen squamifrons</i>	Heard Island	M/F	48	4sm + 44a
<i>Lindbergichthys nitzops</i>	Chiuchia Bank	M/F	48	4sm + 44a
<i>Notothenia microlepidota</i>	and Heard Island	-	26	24sm + 2sm
<i>Notothenia cyanobranchia</i>	Campbell Island	M/F	48	4m + 2sm + 42a
<i>Notothenia rossii</i>	Kerguelen	-	24	24m
<i>Notothenia coriiceps</i>	South Georgia	M/F	24	22m + 2sm
<i>Paranotothenia magellanica</i>	Kerguelen	-	22	22m
<i>Trematomus scotti</i>	Durville Island	-	22	18m + 2sm + 2a
<i>Trematomus eulepidotus</i>	King George Isl.	-	22	18m + 4sm
<i>Trematomus lepidorhinus</i>	Signy Island	M/F	26	24m + 2a
<i>Trematomus bernacchii</i>	Kerguelen	F	50	4m + 2 - 4sm + 42 - 44a
<i>Trematomus pennellii</i>	Weddell Sea	M/F	24	8m + 14sm + 2a
<i>Trematomus hansonii</i>	Prydz Bay	M/F	24	20m + 4sm
<i>Pleuragramma antarcticum</i>	Weddell Sea	F	48	4m + 44a
	Weddell Sea	M/F	48	4m + 44a
	South Shetland Isl.	-	48	2m + 46a
	Weddell Sea	M	48	2m + 2sm + 44a
	Weddell Sea	M/F	32	M: 9m + 2sm + 17a + 4B
	South Shetland Isl.	-	48	2m + 4sm + 42a
	Weddell Sea	F	48	2m + 4sm + 42a
	Weddell Sea	M/F	48	8m + 22sm + 8a

### **Nototheniidae**

Unlike Channichthyidae, this family exhibits a high degree of karyotypic diversity (Table I). From the chromosome morphological point of view, karyotype appears mostly specific, except for very close species such as *Dissostichus eleginoides* and *D. mawsoni*, *N. squamifrons* and *N. kempi*, etc. In most cases where species have been studied in different geographical sectors, no intraspecific variation was observed, except for *Trematomus eulepidotus* (Prydz Bay and Weddell Sea) and *T. bernacchii* (South Shetland and Weddell Sea), which needs to be verified by additional sampling.

Concerning the karyo-evolutionary mechanisms followed by these numerous species, at least three types can be distinguished:

a Chromosome number of  $2n = 48$  conserved; chromosome size conserved; FN slightly increased; minor restructuring involving pericentric inversions.

b Important decrease in the chromosome number (to 22); chromosome size increased, together with the proportion of m-sm types; FN unchanged or slightly modified; major restructuring involving centric fusions between acrocentrics.

c Chromosome number of  $2n = 48$  conserved; chromosome size moderately increased; FN highly increased; possible combination of pericentric inversions and probably duplications.

Considering the cladogram proposed by Balushkin (1984) for the possible relationships among subfamilies of nototheniid fish, our results are in accordance with the primitive position of Eleginopsinae (karyo-evolutionary mechanism of type a), but the subfamilies Nototheniinae ( $2n = 22-50$ ; FN = 44-60) and Trematominae ( $2n = 32-50$ ; FN = 39-58) both include species karyotypes that are close to the ancestral condition as well as derived ones, as if they had been developed independently. In the scope of a cladistic analysis (in prep.), we are trying to interpret those changes in order to select apomorphic characters, sort and rank them, and add them to other features such as osteological, morphological or physiological characteristics.

The karyotype of *Trematomus pennellii* also suggests that some nototheniid species may have developed heteromorphic sex chromosomes as well as Channichthyids, thus providing an additional apomorphic character to be taken into account in phylogenetic studies.

### **Bathydraconidae**

This family comprises ten genera and 15 species: seven genera (7 species) have been now karyotyped (Table I). Diploid numbers range between  $2n = 20$  and  $2n = 48$  with FN = 40-58.

In the group having FN = 40-42, *Bathydraco marri* exhibits a diploid number of 38 chromosomes with only two pairs of large metacentrics, possibly originating from centric fusions between acrocentrics. This species might have differentiated sex chromosomes that could belong to the system XX/XY<sub>1</sub>Y<sub>2</sub>, but this assumption needs to be verified by further studies on additional specimens of both sexes. This heteromorphism may also exist in male karyotypes of *Racovitzia glacialis* that have a very close formula  $2n = 36$ , FN = 40, with a predominance of acrocentrics. In the karyotype of *Prionodraco evansii* ( $2n = 20$ , FN = 40; Prirodina, pers. comm.) all chromosomes are metacentric and the occurrence of differentiated sex chromosomes has not been recorded.

In the group having FN = 44-58, *Psilodraco breviceps* (Prirodina, pers. comm.) has the most primitive formula ( $2n = 48$ , FN = 48) where all chromosomes are acrocentric. *Cygnodraco mawsoni* ( $2n = 44-46a$ ) appears scarcely more derived. *Gerlachea australis* and *Parachaenichthys georgianus* ( $2n = 48$ ) (Prirodina, pers. comm.) also have primitive formulae highly dominated by acrocentrics.



In this family, the chromosome numbers and formulae exhibit a strong variability, involving several kinds of rearrangements (Robertsonian translocations and pericentric inversions) and possibly duplications. These require further detailed investigation and should provide valuable characters for a phylogenetic analysis.

#### Artedidraconidae

This family comprises two monotypic genera, *Dolloidraco* and *Histiodraco*, the karyotypes of which are still unknown, and two polytypic genera, *Artedidraco* and *Pogonophryne*, the taxonomy of the latter remaining controversial. Unfortunately, our first results tend to show that in this family, karyotype ( $2n = 46$ ) is more generic than specific. Species karyotyped for the genus *Artedidraco* differ from those examined for the genus *Pogonophryne* by a single pair, acrocentric in the first and submetacentric in the second. These first results seem to indicate that karyotype is not a powerful criterion for taxonomic studies of the group. Moreover, a comparison with the karyotype of the single species karyotyped in the closest family (Harpagiferidae), *Harpagifer antarcticus* ( $2n = 48$ , Signy Islands, Ozouf-Costaz, unpublished) shows a very similar formula with only a single additional pair of acrocentrics.

### CONCLUSION

The karyotypes of the species studied during EPOS cruise are sufficiently different to enable an estimation of the global value and variability of the karyotypic criteria to be seen as an evolutionary character in each family.

In the Nototheniidae, the Channichthyidae and the Bathydraconidae, the intraspecific variability of the karyotype is not significant, at least in the species studied so far, but the interspecific and/or intergeneric variability shows a sufficient number of numerical and morphological transformations which lead to a tentative first interpretation of the karyo-evolutionary mechanisms. In these three families, further studies are required to clarify the different processes involved in the differentiation in species that have multiple sex chromosomes.

It is more difficult to draw conclusions about the Artedidraconidae, as the karyotypes have only been studied in two genera (8 species). Further research will thus be needed.

It would be of interest to substantiate these results qualitatively, by collecting the karyotypes from the missing sexes of some species and of rarer species which we were not able to keep alive, such as those of the following genera: *Aethotaxis* and *Pagothenia* (Nototheniidae), *Dacodraco* (Channichthyidae), *Akarotaxis* (Bathydraconidae), etc. From a quantitative point of view, a greater number of samples per species and the use of an efficient mitotic stimulator on these fish would produce a sufficient proportion of metaphase spreads to enable a more detailed study on the chromosome structure and karyotype transformations.

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### REFERENCES

- ANDERSEN N.C., 1984. - Genera and subfamilies of the family Nototheniidae (Pisces, Perciformes) from the Antarctic and Subantarctic. *Steenstrupia*, 10 (1): 1-34.



- ANDERSEN N.C. & J.C. HUREAU, 1979. - Proposition pour une nouvelle classification des Nototheniinae (Pisces, Perciformes, Nototheniidae). *Cybium*, 3ème sér.(6): 47-53.
- BALUSHKIN A.V., 1982. - Classification of trematomine fishes of the Antarctic. In: Biology of shelf zones of the world ocean, 2. Izd. DVNTs SSSR, Vladivostok: 9-10 (in russian).
- BALUSHKIN A.V., 1984. - Morphological bases of the systematics and phylogeny of the nototheniid fishes. USSR Academy of Sciences. Zoological Institute, 140 pp.
- DEWITT H.H., 1971. - Coastal and deep-water benthic fishes of the Antarctic. *Antarctic Map Folio*, Ser. 15: 1-10.
- DEWITT H.H., HEEMSTRA P.C. & O. GON, 1990. - Nototheniidae. p. 279-331. In: Fishes of the Southern Ocean (Gon O. & P.C. Heemstra, eds). J.L.B. Smith Institute of Ichthyology, Grahamstown, 462 pp.
- DOUSSAU DE BAZIGNAN M. & C. OZOUF-COSTAZ, 1985. - Une technique rapide d'analyse chromosomique appliquée à sept espèces de poissons antarctiques. *Cybium*, 3ème sér., 9(1): 57-74.
- EKAU W., 1990. - Demersal fish fauna of the Weddell Sea, Antarctica. *Antarctic Science*, 2(2): 129-137.
- HOWELL W.M. & B.A. BLACK, 1980. - Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a one step method. *Experientia*, 36: 1014-1015.
- HUREAU J.C., 1985. - Notothenioidei. p. 244-284 and 323-385. In: FAO species identification sheets for fishery purposes. Southern Ocean (CCAMLR Convention Fishing Areas 48, 58 and 88) (Fischer W. & J.C. Hureau, eds). FAO, Rome, 2: 233-471.
- HUREAU J.C., 1986. - Relations phylogénétiques au sein des Notothenioidei. *Océanis*, 12 (5): 367-376.
- IWAMI T., 1985. - Osteology and relationships of the family Channichthyidae. *Mem. Natl. Inst. Polar Res. Tokyo*, Ser. E, 36: 1-69.
- LEVAN A., FREDGA K. & A.A. SANDBERG, 1964. - Nomenclature for centromeric position of chromosomes. *Hereditas*, 52: 201-220.
- MORESCALCHI A., HUREAU J.C., OLMO E., OZOUF-COSTAZ C., PISANO E. & R. STANYON (in press). A multiple sex-chromosome system in Antarctic ice-fishes. *Polar Biology*.
- MORESCALCHI A., PESTARINO M., PISANO E., STANYON R., STINGO V. & M.A. MORESCALCHI, 1991. - Preliminary data on the cytogenetics of Nototheniid fishes. p. 201-213. In: Proc. 1st. Meet. Biol. Antarctica: Rome 1989. (Battaglia B., Biosol P.M. & M. Varotta, eds). Martello ed., Milano.
- OHNO S., 1974. - Protochordata, Cyclostomata and Pisces. In: Animal cytogenetics 4 Chordata 1 (John B., ed.). Gebruder Borntraeger, Berlin Stuttgart, 92 pp.
- OZOUF-COSTAZ C., 1987a. - Karyotypes of *Chaenodraco wilsoni* and *Chionodraco myersi* (Channichthyidae) from Prydz Bay, Antarctica. *Copeia*, 2: 503-505.
- OZOUF-COSTAZ C., 1987b. - Apport de la caryologie aux études taxinomiques et phylogéniques des poissons. Actes du colloque sur la Recherche française dans les Terres Australes, Strasbourg: 155-163.
- OZOUF-COSTAZ C., 1990. - Programme de cytogénétique des poissons. Les rapports des campagnes à la mer à bord du "Marion-Dufresne", MD42/SIBEX du 3 janvier au 18 février 1985. *Les publications de la Mission de Recherche des T.A.A.F.* n° 85,01: 183-190.
- OZOUF-COSTAZ C. & M. DOUSSAU DE BAZIGNAN, 1987. - Chromosome relationships among 15 species of Nototheniidae. p. 413-419. In: Proc. Vth Cong. Europ. Ichtyol., Stockholm, Sweden (Kullander S.O. & B. Fernholm, eds). Depart. Vert. Zool., Swedish Museum of Natural History, Stockholm.
- PHAN V.N., GOMES V. & H. SUZUKU, 1986. - Estudos Citogeneticos de Peixes Antarticos. I. Cariotipos de *Notothenia gibberifrons* (Lönnberg, 1905), *Trematomus bernacchii* (Boulenger, 1902) e *T. hansonii* (Boulenger, 1902), Perciformes, Nototheniidae. *An. Acad. Brasil. Ciênc.*, 58 (Supl.): 23-27.
- PHAN V.N., GOMES V., SUZUKI H. & M.J. DE PASSOS, 1987. - Karyotypes of two antarctic fishes, *Notothenia gibberifrons* and *Notothenia coriiceps neglecta*. *Japan. J. Ichtyol.*, 33(4): 384-387.
- PRIRODINA V.P., 1984. - Karyotypes de trois espèces de Nototheniidae. *Biol. Morya*, 3: 74-76 (en russe).
- PRIRODINA V.P., 1986. - Karyotypes of *Cottoperca gobio* (Bovichthyidae, Notothenioidei) as compared to karyotypes of other Notothenioidei. Morphology and distribution of fishes of the Southern Ocean. *Proc. Zool. Inst. Leningrad*, 153: 67-71 (in Russian).

- PRIODINA V.P., 1989. - New karyological data on three species of white-blooded fishes (Notothenioidae, Channichthyidae). *Proc. Zool. Inst., USSR Acad. Sci.*, 201: 66-71 (in Russian).
- PRIODINA V.P. & A.V. NEYLOV, 1984. - Les formules chromosomiques de deux espèces de poissons du genre *Notothenia* (Famille Nototheniidae) provenant de l'Ouest Antarctique. *Tr. Zool. Inst.*, 127: 32-37.
- VOSKOBOINIKOVA O.S., 1986. - Evolution of the visceral skeleton and phylogeny of the Nototheniidae. Morphology and distribution of fishes of the southern ocean. *Proc. Zool. Inst., Leningrad*, 153: 46-66 (in Russian).

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